Capillary Electrophoretic Behaviors of Pharmacologically Active Xanthones from *Securidaca inappendiculata* with β-Cyclodextrin as a Buffer Additive

Tao Bo[1], Yongfa Huang[2], Xuedong Yang[3], Ke An Li[1], Huwei Liu[1,*], and **Lizhen Xu[3]**

[1]Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing, 100871, P.R. China; [2]Jiu Zhi Tang Inc., Chang sha, 410008, P.R. China; and [3]Institute of Medicinal Plant Development, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, 100094, P.R. China

Abstract

The capillary electrophoretic (CE) behaviors of ten xanthones in the presence of β-cyclodextrin (CD) are investigated, and apparent analyte-selector binding constants between β-CD and the xanthones in the CE running buffer are calculated to elucidate the migration order. Also, the separation selectivity with β-CD additive is compared with that of sulfated β-CD additive. It is indicated that β-CD can greatly change the separation selectivity of xanthones, and the electrophoretic behaviors of xanthones are rather different when using β-CD from that when using sulfated β-CD as an additive.

Introduction

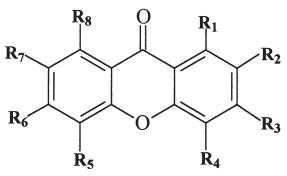
Securidaca inappendiculata Hassk, of which xanthones are the main components, is a traditional Chinese herbal medicine used as an antiinflammatory, antibacterial, and antirheumatism agent (1). As a special class of compounds with strong pharmacological activity (2), all xanthones can be found in the same herbal medicine. A capillary electrophoresis (CE) method has been developed (3) for the separation of xanthones in our lab, which used sulfated β-cyclodextrin (CD) as an additive in the running buffer. In addition, the apparent analyte-selector binding constants of studied xanthones with sulfated β-CD have also been measured (4).

There have been many publications on the analysis of traditional Chinese medicines by CE (5–7), but only a few of them have dealt with the effects of CDs as well as their derivatives on the separation of bioactive components in herbal medicines. In this study, the electrophoretic behaviors of ten xanthones (see Figure 1 for their structures) were further studied by CE using a borate complexing running buffer containing β-CD and methanol as additives. At the same time, apparent analyte-selector binding constants between β-CD and the xanthones were calculated and compared with those between the xanthones and sulfated β-CD.

Experimental

Apparatus and conditions

All separations were performed on an Agilent (Palo Alto, CA) 3D CE system with air-cooling and a diode-array detector. A 58.5-cm × 100-µm-i.d. fused-silica capillary (Ruifeng, Hebei, China) was used with an effective length of 50 cm and the temperature was maintained at 25°C. The other operation conditions were as follows: the applied voltage was 20 kV, UV detection was at 265 nm, and a 50-mbar sample injection was performed for 10 s.



No	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1	HO	MeO	MeO	H	H	H	HO	H
2	MeO	H	H	MeO	H	H	HO	H
3	MeO	HO	H	H	H	H	MeO	H
4	H	H	MeO	HO	H	H	MeO	H
5	MeO	HO	H	H	H	H	HO	H
6	HO	H	H	MeO	H	H	HO	H
7	HO	H	HO	MeO	H	H	HO	H
8	HO	MeO	HO	H	H	H	HO	H
9	HO	H	HO	H	H	H	HO	H
10	HO	MeO	MeO	H	H	HO	HO	H

Figure 1. The chemical structures of ten xanthones.

* Author to whom correspondence should be addressed.

The capillary was conditioned daily by washing first with 0.5M sodium hydroxide (10 min), then with water (10 min), and finally with the running buffer (15 min). Between consecutive analyses, the capillary was flushed with 0.5M sodium hydroxide (1 min), then with water (2 min), and finally with the running buffer (3 min) in order to improve the migration time and peak-shape reproducibility.

Reagents and materials

The xanthones were provided by the Institute of Medicine Plant Development (Beijing, China). All chemicals were of analytical-reagent grade: boric acid, sodium hydroxide, and methanol were from Beijing Chemical Factory (Beijing, China), and pure water prepared by Milli-Q system (Millipore, Bedford, MA) was used for all buffer solutions. β-CD was kindly provided by Bioanalytical Systems (West Lafayette, IN).

Buffer and standard solutions

The buffer solutions containing certain amounts of borate and β-CD were adjusted to the desired pH with 1M NaOH, filtered through a 0.45-µm membrane filter, and degassed by ultrasonication for approximately 10 min before use. A standard solution of approximately 20 ppm of each xanthone was prepared in methanol, filtered, and degassed with the same procedure as used for the buffer solutions.

Results and Discussion

Optimization of electrolyte solutions

Effect of pH

A series of borate buffers with the same borate concentration (30mM) but at different pH values (8.5–10.5) under 20-kV applied voltage and a 25°C temperature was tested in initial CE experiments to resolve the xanthones. As shown in Figure 2, the migration times of all of the xanthones increased with the increase of pH from 8.5 to 10. It could be well explained by the fact that higher pH produced greater ionization of the phenolic hydroxyl groups resulting in greater mobilities of these analytes in the opposite direction to the electroosmotic flow (EOF), although the EOF also increased at higher pH. However, the migration times of all the xanthones decreased above pH 10.5, showing that the increase in EOF is dominant at higher pH. It can be observed that peak shapes and resolutions of the compounds were poor at pH 8.5–9.5 and improved at pH 10. Consequently, pH 10 was chosen for further optimization, at which xanthones 1 and 5–10 were separated with reasonable resolution (as indicated in Figure 3) and xanthones 2–4 were not resolved at all.

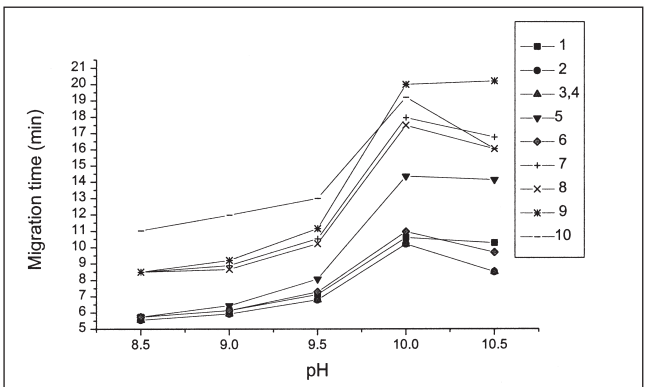


Figure 2. The effect of pH on the separation of xanthones. The curves are correspondent to those in Figure 1.

For increasing UV detection sensitivity for the further determination of xanthones in the real samples, we used a 100-µm-i.d. capillary in the experiments instead of a 50-µm-i.d. capillary. The experiments showed that a 100-µm-i.d. capillary exerted rather different results from that obtained using a 50-µm-i.d. capillary as previously reported (4). Especially at pH 8.5–9, the peak shape and separation selectivity for these xanthones were significantly better when using a 100-µm-i.d. capillary than using a 50-µm-i.d. capillary.

In an attempt to improve the separation, 10% methanol was added into the borate buffer system, but unfortunately no obvious improvement was obtained. In addition, the decrease in EOF with the addition of methanol resulted in analytical times as long as 35 min for these xanthones.

Effect of β-CD concentration

As it is well known, different CDs (especially β-CD and its derivatives) have been successfully used as chiral selectors in enantioseparations, but in this study β-CD was added into the buffer system in order to enhance the separation and investigate the CE behaviors of the xanthones. The effect was studied with a 30mM borate buffer at pH 10, 20 kV applied voltage, and a 25°C temperature. Several electrolyte solutions containing different β-CD concentrations ranging from 5 to 20mM were tested. It was speculated from their structures (see Figure 1) that the xanthones could form inclusion complexes with β-CD. Because of

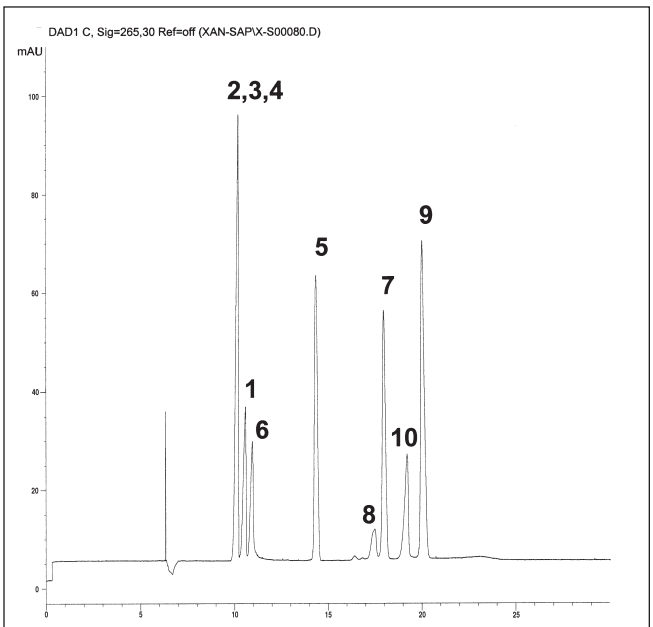


Figure 3. The electropherogram of xanthones at pH 10. The peak numbers are correspondent to those in Figure 1.

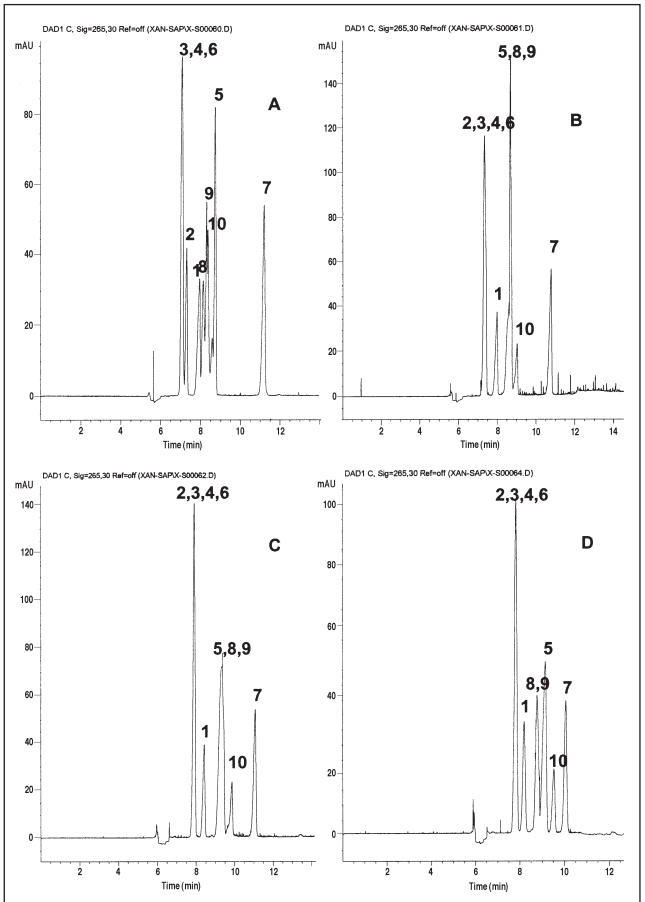


Figure 4. The electropherograms of xanthones at different β-CD concentrations: (A) 5mM, (B) 10mM, (C) 15mM, and (D) 20mM. See Figure 1 for peak identification.

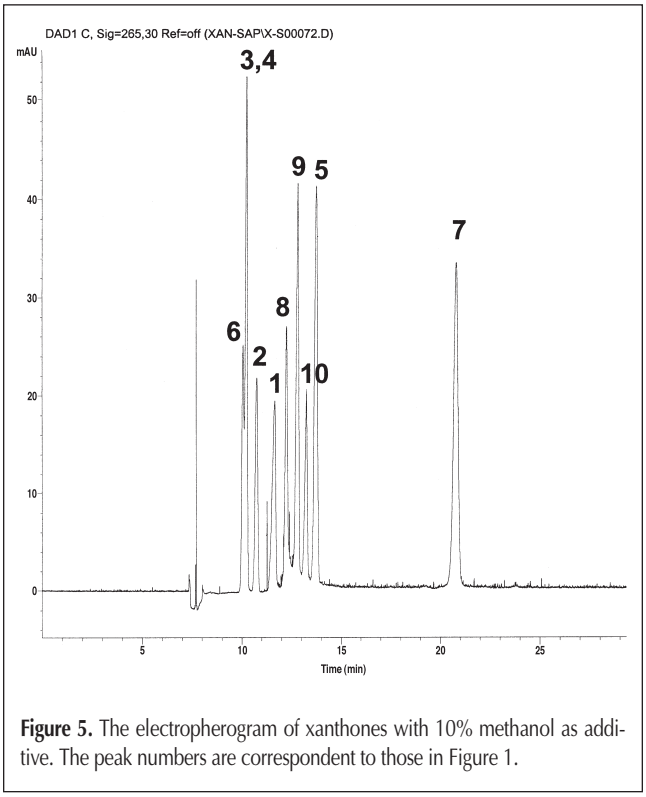


Figure 5. The electropherogram of xanthones with 10% methanol as additive. The peak numbers are correspondent to those in Figure 1.

the different hydrophobic constants, the inclusive effects were different. Also, the different number and position of the phenolic hydroxyl groups of the xanthones may have induced different electric and hydrogen bond effects with the hydroxyl groups of β-CD, thus the addition of β-CD resulted in the great changes of the CE behaviors of the xanthones. It was indicated that the migration order of the xanthones changed dramatically with the increase of β-CD concentration and the migration times did not change significantly. At a 5mM β-CD concentration, the best separation trend was obtained, as is shown in Figure 4.

As compared with sulfated β-CD, the separation selectivity of the xanthones extremely varied with β-CD as an additive. When sulfated β-CD was used the xanthones showed better resolutions (4) because sulfated β-CD is a counter-migrating complexing agent and combines the properties of a CD and a surfactant by providing a hydrophobic cavity and a negative charge. Thus, it can form inclusion complexes with xanthones, and the electrophoretic mobilities of these complexes are in the direction opposite to the EOF.

For optimizing the separation, 10% methanol was added into the buffer containing 30mM boric acid (pH 10) and 5mM β-CD, thus showing that the addition of methanol greatly improved the separation. Xanthones 1, 2, 5, and 7–10 were basically separated, and compound 6 could be discriminated with compounds 3 and 4, but compounds 3 and 4 coeluted because of their extremely similar structures (see Figure 5).

Calculation of apparent analyte-selector binding constants

Knowledge of binding constants when inclusive complexation occurs is of interest in order to appreciate the extent of an analyte inclusion into the cavity of a CD. A theoretical model correlating mobility to the concentration of the CD selector was developed by Wren and Rowe (9,10). Calculating binding constants between xanthones and β-CD helps to elucidate the separation mechanism. If there is a rapid inclusive complexation balance between the analyte and selector in a solution (with a binding ratio of 1:1), the binding constant could be determined by the expression:

$$[S] + [L] \rightarrow [S-L]$$
$$K = [S-\mathrm{L}]/[S][L] \qquad \text{Eq. 1}$$

$$\mu_i = \frac{[S]}{[S]+[S-L]}\mu_f + \frac{[S-L]}{[S]+[S-L]}\mu_c \qquad \text{Eq. 2}$$

The following equation can be elucidated from equations 1 and 2:

$$\mu_i = \frac{\mu_f + \mu_c K[L]}{1 + K[L]} \qquad \text{Eq. 3}$$

where K is the binding constant, L is the equilibrium concentration of uncomplexed ligand, S is the concentration of analyte, $[S-L]$ is the concentration of the analyte–selector, μ_f and μ_c are the electrophoretic mobilities of the free and complexed solute, respectively, and μ_i is the solute mobility at the ligand concentration L.

The mobility of the complex, μ_c, must be measured in order to use equation 3 directly to estimate binding constants. Although it approaches the solute mobility at a very high concentration of β-CD, sometimes its measurement is difficult or impossible because of the difficulty of finding suitable CD makers and reaching saturating conditions. In addition, an increase in the CD concentration leads to some changes in the electrophoretic mobility (because of changes in, for example, the buffer viscosity and EOF), which are not related to the selector–selectivity and binding ability. This can be a source of systematic error in this technique. In order to avoid this problem, equation 3 can be rearranged to the following plotting form, which does not require the direct measurement of μ_c:

$$\frac{[L]}{\mu_f - \mu_i} = \frac{1}{\mu_f - \mu_c}[L] + \frac{1}{(\mu_f - \mu_c)K} \qquad \text{Eq. 4}$$

In order to calculate the β-CD–xanthone binding constants, the electrophoretic mobilities for the xanthones were measured based on electropheregrams in Figure 4 at a different concentration of β-CD. The binding constant can be calculated by the slope and intercept of equation 4. Table I summarizes the calculating results. In most cases, good correlation coefficients between L and $L/(\mu_f - \mu_i)$ were achieved. However, the binding constant of β-CD and xanthone 7 lacked the physical meaning because of its negative intercept. The reason is probably that the binding ratio of β-CD and xanthone 7 was not 1:1.

The observation that xanthone 1 had the lowest binding constant can be explained by the fact that the higher density electron on its 2,3-dimethoxyl group has strong electric repulsion with that on the hydroxyl groups of β-CD, thus decreasing the stability of inclusion complexes. Because of the lower binding constant of xanthone 1 (which induced the little shift in migration time with the increase of β-CD concentration), the binding constant calculation of xanthone 1 produced higher deviation and the correlation coefficient of xanthone 1 was much lower than others. However, xanthone 10 with 2,3-dimethoxyl groups had the highest binding constant. It is speculated that xanthone 10, only catechol xanthone, first complexes with borate and then complexes with β-CD. This three-unit complex is more stable than other complexes and greatly offsets the negative contribution of electric repulsion. However, at the higher borate concentration (200mM) for xanthone 10, the correlation coefficient was much lower. It is speculated that borate competes to form an analyte–selector complex with β-CD instead of forming more-stable three-unit complexes, which was also observed in the experiment of calculating binding constants between sulfated β-CD and the xanthones. In addition, xanthones 3 and 4 are positional isomers and have the same binding constants under our separation conditions, producing a coeluted peak.

Compared with β-CD, sulfated β-CD showed completely different binding constants with the xanthones (4). In general, the xanthones containing a 1-OH group (xanthones 6–9) showed higher values for their apparent binding constants with sulfated β-CD versus xanthones without the 1-OH moiety. This 1-OH exhibited a stronger ability to form a hydrogen bond than others because carboxide has stronger electric attraction, resulting in the great decrease of the electron cloud density around 1-hydrogen atomic nuclear. Therefore, it was easier for the 1-OH of xanthones 6–9 to form a hydrogen bond with the oxygen atom of the sulfonic acid group bonding to β-CD than others. It is believed that hydrogen-bond effects play a very important role in the molecule recognition of the inclusive effect between sulfated β-CD and xanthones. The reason for this is that the binding constant of xanthone 1 and sulfated β-CD is very low and similar to what is described about β-CD. For β-CD, the results mentioned previously were not observed. It is speculated that the electric effect ability of the hydroxyl group on β-CD was much weaker than that of the oxygen atom of the sulfonic acid group bonding to β-CD. Thus, hydrophobicity was the most important factor in the molecular recognition of the inclusive effect of β-CD, which was beyond the electric effect. Perhaps, it can well explain why the binding constants of β-CD were rather different from those of sulfated β-CD.

Table I. Apparent Binding Constants of β-CD and the Ten Xanthones*

Xanthone	Binding constant (L/mol)	Linear regression equation	Correlation coefficient
1	16.19	$y^{\dagger} = 5.960 + 0.0965x^{\ddagger}$	0.9002
2	246.0	$y = 1.2565 + 0.30910x$	0.9900
3	447.4	$y = 0.7415 + 0.33172x$	0.9822
4	447.4	$y = 0.7415 + 0.33172x$	0.9822
5	177.7	$y = 1.049 + 0.1864x$	0.9115
6	659.6	$y = 0.3815 + 0.25164x$	0.9921
7	–7.45	$y = 4.804 - 0.0358x$	0.9970
8	375.4	$y = 0.371 + 0.13928x$	0.9740
9	386.0	$y = 0.3095 + 0.11946x$	0.9844
10	934.2	$y = 0.1704 + 0.15918x$	0.9912

* See the Experimental section for conditions.
† x denotes L (mmol/L).
‡ y denotes $[L]/(\mu_f - \mu_i)$ [(mmol • s • kV)/(L • cm² • 10³)]

Conclusion

In this study, the CE behaviors of ten xanthones were investigated by using borate buffers at different pH values with the addition of β-CD and methanol. Apparent analyte–selector binding constants between β-CD and xanthones were calculated for further elucidating the separation mechanism. The results were compared with that of sulfated β-CD as an additive. The experiments showed that the separation selectivity and binding constants between the two kinds of β-CDs are obviously different. The electric effect is a key factor in the molecule recognization of the inclusive effect. This observation is essential for understanding the separation process of xanthones.

Acknowledgments

The authors would like to thank Agilent Inc. for providing the 3D CE system and Bioanalytical System for the presentation of β-CD. This study was supported by the National Scientific Fund Committee of China, Grant No. 20275001.

References

1. Jiangsu Institute of Botany, Chinese Academy of Medical Sciences, and Kunming Institute of Botany. *Xinhua bencao gangyao*. Shanghai Science and Technology Press, Shanghai, China, Vol. 1, 1988, pp. 292–93.
2. A. Marston, M. Hamburger, and I. Sordat-Diserens. Xanthones from *Polygaly nyikensis*. *Phytochemistry* **33(4):** 809–12 (1993).
3. T. Bo, X.D. Yang, F. Gao, H.W. Liu, K.A. Li, and L.Z. Xiu. Optimized separation of pharmacologically active xanthones from *Securidaca inappendiculata* by capillary electrophoresis. *Chin. Chem. Lett.* **13(3):** 269–72 (2002).
4. T. Bo, X.D. Yang, F. Gao, H.W. Liu, K.A. Li, and L.Z. Xiu. Optimized separation of pharmacologically active xanthones from *Securidaca inappendiculata* by capillary electrophoresis. *Chromatographia* **55(3/4):** 217–23 (2002).
5. B.L. Lee and C.N. Ong. Comparative analysis of tea catechins and theaflavins by high performance liquid chrematography and capillary electrophoresis. *J. Chromatogr. A* **881(1-2):** 439–47 (2000).
6. H. Long, J.J. Yang, H.W. Liu, T.S. Wang, A.J. Huang, and Y.L. Sun. Analysis of chlorogenic acid in herbal medicines by capillary electrophoresis. *Chin. Chem. Lett.* **9(10):** 941 (1998).
7. X.D. Su, T. Bo, R.K. Li, K.A. Li, and H.W. Liu. Determination of isoquinoline alkaloids in thalictrum herbal drugs by non-aqueous capillary electrophoresis. *Chromatographia* **55(1/2):** 63–68 (2002).
8. S.A.C. Wren and R.C. Rowe. Theoretical aspects of chiral separation in capillary electrophoresis. *J. Chromatogr.* **609:** 363–67 (1992).
9. S.A.C. Wren and R.C. Rowe. Theoretical aspects of chiral separation in capillary electrophoresis. *J. Chromatogr.* **603:** 235–41 (1992).

Manuscript accepted March 6, 2003.